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Some Phytochemical Properties and Effect of Fermentation on the Seed of *Jatropha curcas* L.

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ABSTRACT

The present study was undertaken to investigate the effects of microbial fermentation of *Jatropha curcas* L. seed (Barbados nut or Physic nut) or (Lapalapa) in Yoruba Language which are nutritionally underutilized. The study also sought to screen for some phytochemicals, determine the proximate, anti nutrient and some antioxidant properties of the fermented and non-fermented seed powder of *Jatropha curcas* L. to establish the multi-purpose usefulness of the seed, as well as determine the physicochemical properties of the extracted oil. The results showed that fermentation caused slight decrease in the carbohydrate, fat and crude fiber contents but enhanced the protein and ash contents of the seed while the antinutrient contents (tannins, phytate, oxalate and trypsin inhibitor) was observed to decrease as a result of fermentation. The antioxidant properties also witnessed a decrease in percentage 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical inhibition, amount of total phenolic compounds and super oxide anion radical inhibition as a result of fermentation. The phytochemical screening of methanolic and ethyl acetate extracts showed positive results for some secondary metabolites like tannins, glycosides, steroids, saponins and alkaloids in the unfermented seeds but slightly positive for the fermented seeds. The physicochemical analyses of the oil such as acid value, iodine value, saponification value, peroxide value and free fatty acid of unfermented seed appear to be higher than the fermented seed. The results of the analyses showed that the seed of *Jatropha curcas* possessed good antioxidant potentials and if fermented could enhance its nutritional qualities and reduce its anti-nutrients properties.

Key words: Proximate, antinutrients, antioxidant, underutilized, fermentation

INTRODUCTION

Jatropha is native to Central America (Fairless, 2007) and has become naturalized in many tropical and subtropical areas, including India, Africa and North America. Originating in the Caribbean, Jatropha was spread as a valuable hedge plant to Africa and Asia by Portuguese traders. Jatropha species belong to the family Euphorbiaceae and used in traditional folk medicine to cure various ailments in Africa, Asia and Latin America (Chopra *et al*., 1956; Martinez, 1959; Burkill, 1994). *Jatropha curcas* L. is an ornamental, medicinal and multipurpose shrub that are grown in home-gardens in West Africa and cultivated extensively in Asia, *Jatropha curcas* L. is a drought resistant tropical tree. The oil from its seeds have been found useful for medicinal and veterinary purposes, as insecticide, for soap and candle productions, feedstock and for producing biodiesel (Gübitz *et al*., 1999). The deoiled seed cake of Jatropha is suitable as substrate for enzyme

production by Solid-state Fermentation (SSF) and also supported good bacterial growth and enzyme production as evident by its chemical composition (Mahanta *et al*., 2007). The mean weight of the various species of Jatropha seed provenances was found to be 0.64±0.10 g while the kernel forms a large proportion of the seed and accounts for 61.3±3.1% of the weight and there were large variations in the crude protein, lipid, neutral detergent fiber and ash contents of the kernels though the gross energy of kernels was relatively similar (Makkar *et al*., 1997). Trypsin inhibitor activity, saponins, phytate, lectin activity in the defatted kernels meal of the various species also varied in percentage compositions as tannins, amylase inhibitor, glucosinolates and cyanogens were not detected in the meals various species (Makkar *et al*., 1997). The young leaves may be safely eaten, steamed or stewed. Cooked with goat meat, they are said to advantageously counteract its smell. Pounded leaves are applied near horses' eyes to repel flies in India. The nuts are sometimes roasted and eaten, although they are purgative and are used as a contraceptive in South Sudan, all the parts of the shrubs containing hydrogen cyanide (HCN). The seeds also used as a contraceptive in South Sudan, the oil has been used for illumination, making Turkey red oil also called sulphonated (or sulfated) castor oil, is the only oil that completely disperses in water which is obtained by adding sulfuric acid to pure Jatropha oil (Achten *et al*., 2007, 2008).

The ashes of the roots which in addition to HCN, contain rotenone and are used as a salt substitute. The bark is used as a fish poison while the latex strongly inhibits the watermelon mosaic virus and the sap stains linen and sometimes used for marking.

MATERIALS AND METHODS

Sample preparation: *Jatropha curcas* L. seeds used in this investigation was obtained from Ilorin, Kwara State, Nigeria in year 2008, dried and ground to powder. Microbial fermentation of *Jatropha curcas* L. seed powder was carried out using pure strain of *Rhizopus oryzae* obtained from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria, which was isolated from *Jatropha curcas* L). After fermentation, the samples were oven-dried overnight at 37°C and made to powder.

Determination of proximate and antinutrients contents: The Proximate compositions (crude protein, crude fat, crude fibre, ash, moisture and carbohydrate) were determined according to the methods of AOAC (1990).

The phytate content was determined by the method of Wheeler and Ferrel (1971) based on the ability of standard ferric chloride to precipitate phytate in dilute HCl extracts of the sample.

The tannin contents of fermented and the unfermented samples were determined using the method of Makkar *et al*. (1993) which is based on the ability of tannin-like compounds to reduce phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution, the intensity of which was measured with Spectrophotometer at 725 nm which is proportion to the amount of tannins present in the solution.

The oxalate was determined by the AOAC (1990) method based on the reaction of 0.05 N $KMnO_4$ with the oxalic acid in the solution.

While the trypsin inhibitor was determined, according to the method of Kadeka *et al*. (1974) which was based on the extent to which a portion of an aqueous extract of the seed inhibited the action of trypsin on N-Benzoyl-DL-Arginine-P-Nitroanilide Hydrochloride (BAPNA) as substrate and the absorbance was read at 410 nm.

Determination of 1, 1-Diphenyl –2-Picryl Hydrazyl (DPPH) free radical scavenging ability: The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical was evaluated as described by Gyamfi *et al.* (1999). Briefly, an appropriate dilution of the extracts (1 mL) was mixed with 1 mL of 0.4 mmol L^{-1} methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm. The DPPH free radical scavenging ability was subsequently calculated with respect to the reference (which contains all the reagents without the test sample).

Determination of total phenol content: The total phenol content of the extracts was determined using the method reported by Singleton *et al.* (1999). Appropriate dilutions of the extracts were oxidized with 2.5 mL of 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated using tannic acid as standard.

Determination of super oxide anion radical scavenging: Super oxide radicals are generated in nicotinamide adenine dinucleotide-phenazine methosulphate (NADH-PMS) systems by oxidation of NADH and assayed by reduction of nitro blue tetrazolium (NBT).

Super oxide radicals scavenging activity was based on the capacity of the extract to inhibit the photochemical reduction of Nitro Blue Tetrazolium (NBT). Superoxide dismutase which catalyzed the neutralization of superoxide was followed after modification of standard method (Martinez *et al.*, 2001) 3.0 mL of 16 mM Tris-HCl buffer (pH 8.0) added to 1.0 mL of 50 μM NBT solution in a test tube, 1.0 mL of 78 μM NADH solution, 1.0 mL aqueous solution of the extracts (300 μg mL^{-1}) and 1.0 mL of 10 μM phenazine methosulphate (PMS) solution. A similar control and blank test tubes were prepared containing all but sample and PMS, respectively. The reaction mixture was incubated at 25°C. The production of blue formation was followed by monitoring the increase in absorbance at 560 nm against blank after 5 min illumination. The entire reaction setup was enclosed in a box lined with aluminium foil. The percentage inhibition of super oxide anion generation was calculated by comparing absorbance values of the control and reaction mixture containing the sample solution.

Phytochemical qualitative analysis of fermented and non-fermented *Jatropha curcas* L. seed powder: Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Test for Tannins: About 0.5 g of the dried powdered samples was boiled in 20 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for Saponin: About 2 g of the powdered sample was boiled in 20 mL of distilled water in a water bath and filtered. Ten milliliter of the filtrate was mixed with 5 mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Alkaloids: Five milliliter of 1% aqueous hydrochloric acid was added to 2 g of the extract in a test tube, heated in a steam bath and filtered; 1 mL of the filtrate was treated with 6-10 drops of Dragendoff's reagent. The presence of creamish precipitate or turbidity after addition was taken for the presence of alkaloid.

Test for steroids: Two milliliter of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 mL H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for cardiac glycosides (Keller-Killani test): Five milliliter of each extracts was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 mL of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides.

A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Extraction and analysis of physicochemical properties of oil from both fermented and non-fermented *Jatropha curcas* L. seed powder: Extraction of oil from both fermented and non-fermented *Jatropha curcas* L. seed powder and analyses of oil for its physicochemical properties (acid value, iodine value, saponification value, peroxide value and free fatty acid) from both fermented and non-fermented *Jatropha curcas* L. seeds were carried out according to standard methods (AOAC, 1990).

RESULTS

The results of *Jatropha curcas* L. seeds for proximate composition from Table 1 revealed that the sample is a good source of protein and rich in fiber content with very high concentration of crude oil. The effect of fermentation on the proximate composition of the seed on dry matter basis therefore showed significant increase in the protein and ash contents but decrease significantly in the crude fat, rude fiber and carbohydrates as seen in Table 1.

As evident from Table 2, the percentage of the studied antinutrient parameters is slightly high for phytate and oxalate but slightly low for tannins, though it is considerably high for the trypsin inhibitor. The effect of fermentation on the seeds however showed a significant decrease in the phytate, tannins, oxalate and trypsin inhibitor.

The percentage inhibition of DPPH and superoxide anion free radicals of the seed was high as the amount of the total phenolic compound was slightly higher but the fermentation of the seeds caused almost fifty percent reduction in these antioxidant properties studied as obtained in Table 3.

The seeds of *Jatropha curcas* L. was analyzed to contain the studied plant secondary metabolites which are of medicinal importance, the however showed a reduction in these component because of fermentation as shown in Table 4.

The physicochemical properties of the oil of the *Jatropha curcas* L. seeds showed the parameters are generally low except saponification value, it is also evident from Table 5 that fermentation caused a decrease in all the studied parameters even in the extracted oil from the fermented sample.

Table 1: Proximate composition of fermented and non-fermented *Jatropha curcas* L. seeds (dry matter basis)

Parameters (%)	Fermented	Non-fermented
Moisture	6.67±0.09^{a}	5.43±0.15^{b}
Carbohydrate	7.83±0.17^{b}	8.57±0.15^{c}
Fat	40.06±0.14^{b}	47.10±0.06^{d}
Protein	26.57±0.05^{c}	24.14±0.05^{d}
Crude fiber	8.20±0.08^{a}	10.03±0.16^{b}
Ash	4.75±0.06^{b}	4.43±0.16^{a}

Values with different superscripts in the same row are significantly different while values with the same superscript in the same row are not significantly different (at 95% confidence level)

Table 2: Some anti nutrient contents of fermented and non-fermented *Jatropha curcas* L. seed powder

Parameters (%)	Fermented	Non-fermented
Phytate	4.10±0.08^{a}	7.20±0.01^{b}
Tannins	0.76±0.01^{c}	0.92±0.02^{d}
Oxalate	3.32±0.02^{a}	6.10±0.08^{c}
Trypsin inhibitor	11.50±0.11^{a}	21.10±0.08^{b}

Values with different superscripts in the same row are significantly different while values with the same superscript in the same row are not significantly different (at 95% confidence level)

Table 3: Some antioxidant properties of fermented and non-fermented *Jatropha curcas* L. seed powder

Parameters (%)	Fermented	Non-fermented
Inhibition of DPPH radical	10.77±0.06^{a}	22.10±0.08^{b}
Amount of total phenolic compounds	1.17±0.06^{c}	3.23±0.09^{d}
Inhibition of super oxide anion radical	6.63±0.12^{e}	10.27±0.09^{e}

Values with different superscripts in the same row are significantly different while values with the same superscript in the same row are not significantly different (at 95% confidence level)

Table 4: Phytochemical screening analysis on fermented and non-fermented *Jatropha curcas* L. seed powder

	Secondary metabolites				
Extract sample	Tannins	Glycosides	Steroids	Saponins	Alkaloids
Methanol					
Fermented	+	+	+	+	+
Non-fermented	++	++	++	++	++
Ethyl acetate					
Fermented	+	+	+	+	+
Non-fermented	++	++	++	++	++

+: Slightly positive; ++: Positive

Table 5: Analysis of physicochemical properties of the extracted oil obtained from fermented and non-fermented *Jatropha curcas* L. seed powder

Parameters	Fermented	Non-fermented
Oil content (%)	35.03±0.12^{a}	41.27±0.12^{a}
Acid value (mg KOH g^{-1})	1.36±0.01^{b}	1.98±0.01^{b}
Iodine value (mg iodine g^{-1})	1.42±0.01^{c}	2.23±0.12^{d}
Saponification value (mg KOH g^{-1})	25.30±0.08^{a}	28.67±0.17^{b}
Peroxide value (m Eq kg^{-1})	0.21±0.01^{b}	0.29±0.005^{c}
Free fatty acid (%)	0.68±0.02^{c}	0.97±0.02^{d}

Values with different superscripts in the same row are significantly different while values with the same superscript in the same row are not significantly different (at 95% confidence level)

DISCUSSION

There were significant differences in the results of the proximate composition of the *Jatropha curcas* seeds as a result of fermentation Table 1, the carbohydrate (7.83±0.17 and 8.57±0.15), fat (7.83±0.17 and 8.57±0.15) and crude fiber (8.20±0.08 and 10.03±0.16) showed slight decrease while crude protein (26.57±0.05 and 24.14±0.05) and ash (4.75±0.06 and 4.43±0.16) contents showed an increase. The decrease in the carbohydrate, crude fat crude fiber values because of fermentation may be due to their utilization and transformation by fermentation microorganism to obtain energy and other cellular activities. This observation is however similar to that obtained by Oladele and Oshodi (2008) apart from crude fiber which showed slight decrease as a result of fermentation which may be due to the pure stain of organism used for fermentation as against their wide type of fermentation. The reasonable fat content of the seed could be found useful in medicinal and veterinary purposes as insecticide, soap production and as fuel substitute (Gübitz *et al*., 1999). The crude protein content of the fermented sample showed an increase, this high protein contents could be attributed to the ability of the microorganism to secret some extra cellular enzymes (proteins) which degrade the materials during fermentation, this results is similar to the observations obtained when pure strain of *Aspergillus niger* was used to ferment maize cobs by Oseni and Ekperigin (2007); ash content of the fermented sample is slightly higher than the raw sample, this increase may be due to contribution by fermentation microorganisms (Oseni and Ekperigin, 2007; Oladele and Oshodi, 2008).

The fermentation of the seed powder also caused a reduction of almost fifty percent in the in phytate, oxalate and trypsin inhibitor and slight reduction in the tannin contents as evaluated in Table 2. These observations are however contrary to the observations of Oladele and Oshodi (2008) as they observed increased phytate and tannin because of fermentation; but in consonance with what earlier obtained for reduced phytate by Oseni and Ekperigin (2007) when pure strain of *Aspergillus niger* was used to ferment maize cobs, it is however possible that the mode of fermentation and the species of organisms involved play crucial roles in the fermentation processes. Processing by either fermentation, moist heating or any other heat treatment has been reported to reduce anti nutrients constituents of plant foods, these offer promise for inclusion of products from plants in animal and fish diets (Makkar and Becker, 1999).

However, both the fermented and non-fermented samples from Table 3 showed reasonably high antioxidant properties but fermentation also caused a decrease of about fifty percent in the percentage of inhibition of free radicals, amount of total phenolic contents and inhibition of super oxide radical. Although, fruits (Sun *et al*., 2002; Wang *et al*., 1996), wines, juices and vegetables have been reported to exhibit antioxidant activity at different degree and that various conventional food processing can bring a loss in their antioxidant contents (Oboh, 2005; Oboh and Akindahunsi, 2004; Amic *et al*., 2003). Many other works of high phenolic antioxidant contents similar to *Jatropha curcas* seeds have been reported like date fruit by Gad *et al*. (2010) showed that the predominant phenolics found in date fruit were very active antioxidants, also the antiradical activity in dates was highly correlated to the phenolic contents (Mansouri *et al*., 2005).

Table 4 also showed the qualitative determination of some basic secondary metabolites in both fermented and non-fermented seed of the *Jatropha curcas* L. seed powder, which might be responsible for antimicrobial activities and therapeutic potential as supported by Matsuse *et al*. (1999) though fermentation of the samples caused reduction in all the secondary metabolites studied.

The physicochemical properties of the seeds oils from Table 5 showed that both oils have good physicochemical qualities, however, fermentation caused reduction in the values of free fatty acids, peroxide, saponification, acid and iodine values. The acid and free fatty acid values used as indicators of the edibility and suitability of the oil for industrial application, the acid value of the oil suitable for edible purposes should not exceed 4 mg KOH g^{-1} (Esuoso and Odetokun, 1995); the low level of the free fatty acid and peroxide values in both fermented and non fermented samples also showed the non spoilage condition of the oil.

CONCLUSION

Since, there was increase in the parameters like protein and ash contents of the seed, but decrease in various parameters like crude fat, crude fiber, carbohydrate; antinutrients (phytate, tannins, oxalate and trypsin inhibitor); antioxidant properties like (inhibition of DPPH, superoxide anion free radicals and amount of total phenolic compounds); phytochemicals (tannins, glycosides, steroids, saponins and alkaloids); physicochemical properties and oil content of *Jatropha curcas* L. seeds, it could be inferred therefore that fermentation could increase the nutrient composition of the seed and reduction in antinutrients hence increase the possibility of utilizing the seeds as feedstock/food and protein supplements for animals for human. However, fermentation could be discouraged for utilizations of the seeds as natural antioxidants industrial utilization of the oil.

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